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The influence of pigmentation patterning on bumblebee foraging from flowers of *Antirrhinum majus*

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Abstract

Patterns of pigmentation overlying the petal vasculature are common in flowering plants, and have been postulated to play a role in pollinator attraction. Previous studies report that such venation patterning is significantly more attractive to bee foragers in the field than ivory or white flowers without veins. To dissect the ways in which venation patterning of pigment can influence bumblebee behaviour we investigated the response of flower-naïve individuals of *Bombus terrestris* to veined, ivory and red near-isogenic lines of *Antirrhinum majus*. We find that red venation shifts flower colour slightly, although the ivory background is the dominant colour. Bees were readily able to discriminate between ivory and veined flowers under differential conditioning, but showed no innate preference when presented with a free choice of rewarding ivory and veined flowers. In contrast, both ivory and veined flowers were selected significantly more often than were red flowers. We conclude that advantages conferred by venation patterning might stem from bees learning of their use as nectar guides, rather than from any innate preference for striped flowers.

Keywords

Anthocyanin, *Antirrhinum majus, Bombus terrestris*, pigmentation, venation

Introduction

Very few flowers are a simple block of a single colour. Patterns of pigmentation are found in many species, and can include colour patterns within a petal or different colours on different petals. These colours can be visible to the human eye or only to an animal with UV-sensitive vision. Pigmentation patterns are thought to be important in animal pollination. Many studies have demonstrated that bees in particular can identify, and are sometimes attracted to, patterned targets over plain targets (Dafni and Giurfa, 1999; Hempel de Ibarra *et al.,* 2001; Heuschen *et al.,* 2005; Shang *et al*., 2011), and that these patterns can increase the speed with which bees locate the nectar reward in a flower (Leonard & Papaj 2011). Such patterns have the potential to aid pollinator orientation during visits; increase flower visibility to the pollinator eye; improve the ease of search image formation; or induce a mating or aggregation response. Common examples of pigmentation patterns include petal spots, bull's eye targets, yellow patches that mimic pollen, and pigmentation over the petal vasculature or veins (venation patterning; often referred to as nectar guides).

Petal spots are the best studied of these pigmentation patterns, and increase attraction of a variety of animals. Populations of *Clarkia xantiana* ssp. *xantiana* are polymorphic for the presence and number of red petal spots and pollinated by three specialist bee species (Moeller, 2005). The different pollinator species forage in a frequency-dependent manner, one choosing the most common morph and the other two choosing the rarer morph (Gottlieb and Ford, 1988; Eckhart *et al*. 2006). The presence of dark spots on the inflorescences of *Daucus carota* ssp. *maxima* has been shown to attract *Musca domestica* flies (Eisikowitch, 1980), although recent reports suggest that these spots play a greater role in reducing parasite infestation than in enhancing pollination (Polte and Reinhol, 2012). The South African daisy *Gorteria diffusa* has black spots on its ray florets and is pollinated by a small bee-fly, *Megapalpus capensis*. Capitula of *G. diffusa* with black spots received more fly visits than capitula from which the spotted ray florets had been removed, as male flies attempt to mate with the spots (Johnson and Midgley, 1997; Ellis and Johnson, 2010).

A central colour distinct from the rest of the flower (a "bull's eye target") is a common feature of many flowers. This particular form of nectar guide has long been known to increase floral attractiveness to a range of pollinators. Bumblebees and honeybees have been shown to discriminate bull's eye spots as little as 2mm in diameter (Lunau *et al.* 2009). In a series of classic experiments with *Delphinium nelsonii*, which has a white centre in a deep blue flower, Waser and Price (1983; 1985) showed that the central colour patch improves foraging efficiency of both bees and hummingbirds by reducing time to find the flower and time to access the reward. Bull's eye targets can also attract pollinators in the same way that yellow pollen-mimicking patches do. Heuschen *et al.* (2005) showed that the central bull's eye found in many radially symmetrical (actinomorphic) flowers appears similar in colour to pollen to the bee's eye. Accordingly, they proposed that this particular pigmentation pattern attracts pollinators and enhances foraging efficiency by providing not just cues to the location of the reward but a mimic of the pollen itself. Yellow patches are also commonly observed on many zygomorphic (bilaterally symmetrical) flowers, mimicking the appearance either of pollen dusted on the flower throat (such as in *Antirrhinum majus*, figure 1) or of readily accessible stamens (such as in *Hardenbergia violacea*, Lunau, 2006).

Venation patterns (lines of pigmentation overlying the vasculature) are common in many groups of flowering plants, including the orchids (Mudalige *et al.* 2003), *Geranium* species and the genus *Antirrhinum* (Shang *et al.* 2011). However, the influence of this particular pattern of pigmentation on pollinator behaviour has received relatively little attention to date. In theory venation patterns could direct pollinators towards the reward, acting like bull's eye nectar guides. A recent study by Leonard and Papaj provides support for this theory, finding that artificial nectar guides consisting of thin radiating lines enhance the speed with which bumblebees find the reward in artificial flowers (Leonard and Papaj 2011). Alternatively venation patterns could increase flower visibility or the ease with which a floral search image is formed. In any of these cases venation patterns would improve foraging efficiency and therefore

be attractive to bees, particularly after repeated exposure has enabled learning of rewarding and easy-to-handle floral forms.

Shang *et al.* (2011) described the molecular basis of venation patterning in *Antirrhinum*, following the identification by Schwinn *et al.* (2006) of the *Venosa (Ve)* locus as responsible for venation. *Ve* encodes a Myb transcription factor that regulates the expression of genes encoding enzymes of anthocyanin synthesis. *Ve* is expressed in the cells surrounding vasculature on the adaxial side, in both epidermis and mesophyll cells of the petals. However, its interaction with the bHLH transcription factor *Delila*, expressed only in the epidermis (Goodrich *et al.,* 1992), is restricted to the epidermal cells overlying veins. Interaction of the two transcription factors is necessary to generate pigmentation, which therefore occurs only in the epidermal cells overlying veins on the adaxial petal surface (Shang et al., 2011). The red patterning in this line therefore follows the pattern of the veins. On the petal lobes it is restricted to the adaxial epidermis, where it is strongest and most dense towards the closed mouth of the flower (Figure 1), but it continues down the corolla tube in the adaxial epidermis marking the route to the nectar that accumulates at the base of the tube.

Antirrhinum is a genus with a complex evolutionary history. It contains approximately 20 species distributed in the Mediterranean region, many of which produce flowers with pale pigmentation but deeply pigmented venation patterns (Wilson and Hudson, 2011; Shang *et al.,* 2011). The common genetic model *Antirrhinum majus* is usually fully pigmented red. Shang *et al.* (2011) and Schwinn *et al.* (2006) demonstrated that the veined phenotype (Ve^{+}) is only clearly visible in this species in a *rosea* mutant background. *Rosea* is a locus encoding two Myb transcription factors that regulate anthocyanin synthesis across the main body of the Antirrhinum flower (Schwinn *et al.,* 2006). In the many species of *Antirrhinum* that are pale with clear venation patterns it is likely that the *Rosea* locus is promoting little background pigmentation but that the *Venosa* locus is strongly regulating anthocyanin synthesis around the veins (Schwinn et al., 2006).

To investigate the functional significance of venation patterns Shang *et al.* (2011) planted out field plots of a set of near isogenic lines of *Antirrhinum majus*, including red, veined, pink, ivory and white. Each plot represented a pairwise comparison of red versus another colour. They scored pollinator visits to each plant type, and concluded that the three species of foraging bumblebees that they observed significantly favoured red flowers over pink, ivory or white in each of their pairwise comparisons. However, they found only a small, non-significant bumblebee preference for red over veined flowers in the red versus veined plot. They concluded that venation patterning, as seen in many wild *Antirrhinum* species, is highly attractive to bumblebee pollinators.

To dissect the ways in which venation patterning of pigment can influence bumblebee behaviour we investigated the response of flower-naïve individuals of *Bombus terrestris* to veined, ivory and red near-isogenic lines of *Antirrhinum majus*. We find that red venation shifts flower colour slightly, but that the ivory background is the dominant colour effect. Bees were readily able to discriminate between ivory and veined flowers under differential conditioning, but showed no innate preference when presented with a free choice of rewarding ivory and veined flowers. In contrast, both ivory and veined flowers were selected significantly more often than were red flowers. We conclude that advantages conferred by venation patterning might stem from bees learning of their use as efficiency-enhancing nectar guides, rather than from any innate preference for striped flowers.

Materials and Methods

Bees

Flower naive bumblebees (*Bombus terrestris* var. *dalmatiens*) were supplied by Syngenta Bioline (Weert, The Netherlands). Housing conditions and bee marking procedures were as described in Dyer *et al.* (2007), with bees fed 30% sucrose solution daily and pollen provided directly to the colony. Colonies were attached via a plastic tube to a 300 × 750 × 1120 mm plywood flight arena with a UV-penetrable Plexiglas® lid. The inside of the arena was painted in a bee-neutral shade of brown. All experiments were performed outside, with natural lighting. As external temperatures were low the colony was incubated with a patio heater and internal heating pad.

Plants

Seeds for three lines of *Antirrhinum majus* were developed at the John Innes Centre. The lines used were JI522, a full red accession described in this manuscript as "red"; AB103[10], homozygous for both the $rosea^{dorsea}$ allele and the ve^{-} allele, and described in this manuscript as "ivory"; and AB104[10], homozygous for the $rosea^{dorsea}$ allele and heterozygous Ve^{+}/ve^{-}. These flowers had the ivory background of the $rosea^{dorsea}$ allele overlain with red venation, and are described in this manuscript as "veined". The ivory and veined lines had been backcrossed to JI522 for at least 3 generations, and are considered isogenic with respect to JI522. Generation of the parents of these lines is described in Shang *et al.* (2011). Plants were grown in 4 inch pots under greenhouse conditions at 23°C in Levington (UK) M3 compost. Supplemental lighting during the growth period was supplied using 400 Osram lamps from Osram, Munich, on a 16 hour light/8 hour dark photoperiod during. Fresh flowers were picked every two days and kept in water before use. Any flowers which showed signs of wilt or damage were not used.

Analysis of flower colour by spectrophotometry

The spectral reflectance function (SRF) of a large spot area of petals was measured with an Ocean Optics (Dunedin, Fl., USA) spectrophotometer (S2000) relative to a calibrated deuterium/halogen radiation source (DH-2000-CAL) and an Ocean Optics polytetrafluoroethylene 100% white diffuse reflectance standard (Dyer and Chittka, 2004). A section of the background painted with Humbrol (UK) no 63 brown paint was also measured using this method. More detailed measurements of the red and ivory areas of veined flowers were taken with an Ocean Optics spectrophotometer (QE65000) relative to a calibrated Deuterium Tungsten Halogen Light Source (DH-2000-BAL) and the same standard. In this case light from the lamp was coupled to a bifurcated optical fiber probe and focused on the petal using an achromatic lens (focal length of 30 mm). The reflected light was then collected from the same lens and sent to the spectrometer. This configuration allows enough spatial resolution to collect signal only from the veined or ivory regions of the petal. The adaxial face of the dorsal petals of five independent flowers of each plant line was measured to obtain a mean spectral reflectance function.

Calculation of flower colour in bee colour space

Colour loci of the flowers were calculated using the hexagon colour space model of Chittka (1992).

Pretraining of bees

Bees were pretrained to feed from an experimental set-up without flowers. A test tube rack was placed in the arena at an angle (at 45 degrees from vertical, similar to the natural angle of Antirrhinum flowers on a plant). Twelve tubes with parafilm covered tops were placed into the rack at random, such that the tubes were 5cm apart. Slits were cut in the parafilm and small Eppendorf tubes with the lids removed were secured in the parafilm. 30% sucrose solution was provided in the Eppendorf tubes.

Differential conditioning to test discrimination between flower types

Differential conditioning was used to determine whether bees could distinguish between ivory and veined lines (Dyer and Chittka, 2004). Six flowers of each type were presented in the holder, arranged in a pseudo-random pattern. The flowers were pushed into the parafilm slit and a small Eppendorf tube

inserted into the flower to hold the reward or punishment. A single trial consisted of releasing a single marked bee from the colony and allowing her to forage until satiated. Ten bees were tested with veined flowers rewarding, and six bees were tested with ivory flowers rewarding, each to 100 choices (usually 10-12 consecutive trials per bee, where the individual bee made multiple consecutive choices within a trial). A choice was counted when the bee landed on a flower and drank. 20 µl of either 30% sucrose (reward) or a 0.12% solution of quinine hemisulphate salt in water (punishment) was present in alternate flower types (Dyer and Chittka, 2004). During a trial, flowers containing sucrose were refilled once the forager had left the flower and had landed on an alternative flower at least 5cm distant from the original flower. Between trials, all flowers were replaced with fresh flowers to minimise the effect of bee scent marks on foraging behaviour. New flowers were positioned in a different arrangement within the array to prevent the bee associating flower type with a specific location.

Innate preferences of bees

To explore innate preferences flower-naïve bees were presented with an array of flowers in the rack. Flowers were placed in the rack in a random pattern and replaced with fresh flowers in a different pattern between foraging bouts. All flowers contained 20 µl of 30% sucrose solution in an Eppendorf tube, which was refilled after a bee visit. Eight red flowers, four veined flowers and four ivory flowers were tested together against a brown background (Humbrol (UK) no 63 brown paint). A brown background was chosen to simulate the bare rocky soil on which most species of *Antirrhinum*, including those with venation patterning, grow in the Mediterranean (Sutton, 1988). Ten bees were tested individually to a maximum of 100 choices (one bee only made 66 choices). A recorded choice was landing on and feeding from a flower.

Results

Red flowers reflect little light, while venation substantially changes total reflectivity

The reflectance spectra of the flowers, shown in figure 2a, indicate that the red flowers reflect very little light. As predicted from their appearance to the human eye they absorb almost all light below 600nm, with a peak of reflection at 700nm. The reflectance spectrum also indicates that they do not reflect UV light. The ivory flowers reflect significantly more light, particularly in the 400-700nm range, appearing white-ish to the human eye. Their absorption of light in the 300-400nm range, due to the presence of UV-absorbing flavones, is not apparent to the human eye but will shift flower colour from white slightly towards blue/green in insect vision.

We analysed the veined flowers using a single large spot to average out red and ivory cells ("veined large spot") and using a smaller spot to quantify reflectivity from just the red ("veined red") and just the ivory ("veined ivory") parts of the petal. The veined ivory spot gave results very similar to the ivory flower. The veined red spot gave a curve of similar shape to the red flower, but with higher reflectivity, presumably because some ivory cells were incorporated in the analysis. The large spot analysis of the overall appearance of the veined flower produced a curve with features of both ivory and red. In the 300-400nm range the veined flowers absorbed even more light than the ivory flowers, as a result of the combined activity of ivory flavones and red anthocyanins over the veins. In the 400--500nm range they reflected less light than the ivory flowers, but more than the red, indicating that the mean contribution of the coloured veins is to reduce the total reflectivity of the flower. There was no dip in the curve in the 500-600nm range, unlike in the red flowers. The reflectance of light at higher wavelengths to the same degree as light in the 400-500nm range produces an overall profile more similar to the ivory flower than to the red, suggesting that the overall colour appearance of the veined flowers will be similar to that of the ivory flowers, to the bee's eye.

Venation pigmentation shifts average flower colour slightly

The colour space hexagon model devised by Chittka (1992) was used to plot the relative colours of the three flower types and the component red and ivory parts of the veined flowers in bee vision (figure 2b). The red part of the veined flowers sits close to the red flowers, and the ivory part of the veined flowers

sits closer to the ivory flowers in the hexagon. However, the averaged appearance of the veined flowers recorded using a larger spot placed them very close to the ivory flowers. Two of the three flower types are over 0.100 hexagon units apart (ivory to red = 0.263, red to veined = 0.231). However, the ivory and veined flowers are only 0.038 units apart, although the individual components of the veined flower are separately much further apart from one another and from the red and ivory flowers. Determined by colour space alone, the bee visual system should be able to discriminate the red and ivory flowers studied here, but the ivory and veined flowers should be difficult to discriminate on the basis of colour alone.

Bees can discriminate between veined and ivory flowers

To see if bees could discriminate veined from ivory flowers, learning behaviour was analysed. The number of correct visits during the first ten choices was compared with the number during choices 51 to 60, using a paired *t*-test. The bees were able to use the differences in pigmentation pattern as a cue to locate and drink from the rewarding flowers (and to avoid drinking from the adverse flowers), whether the reward was presented in veined or ivory flowers (figure 3). When the veined flowers were rewarding, the bees made 49.0 ± 7.06% (± S.E. presented throughout) correct choices during their first ten visits, which significantly increased to 67.0 ± 5.38% between 51 and 60 visits ($t_9 = 2.37$, $p = 0.041$; figure 3a). When the ivory flowers were rewarding, the mean percentage of correct choices made during the first 10 choices was 48.3 ± 8.33%, but 78.3 ± 4.77% after 51 visits ($t_5 = 5.19$, $p = 0.003$; figure 3b). Bees can therefore successfully learn to differentiate between veined and ivory flowers, whichever is rewarding.

Bumblebees have an innate preference for ivory-based flowers over red, but no preference for or against venation

In the free choice experiment equal rewards were provided in all flowers, and the number of red flowers (eight) was balanced against the number of pale flowers (four ivory and four veined), to minimise frequency dependent effects. Bees visiting this array chose red flowers 25.6 ± 4.38% of the time, ivory flowers 34.1 ± 3.33% of the time and veined flowers 40.3 ± 3.29% of the time. Bees visited pale flowers significantly more than 50% of the time ($t_9 = 5.58$, $p = 0.0003$), which demonstrates that they discriminate against red flowers. Within the visits to paler flowers, the preference for veined flowers over ivory was not significant (paired sample t-test, $t_9 = 1.19$, $p = 0.266$).

Discussion

Venation patterns of pigmentation are common in flowering plants, and have long been hypothesised to play a role in pollinator attraction. Our analysis of flower colour indicates that anthocyanic veins are themselves very close to the colour of a red flower, but have little total effect on flower colour from a distance (measured with a large spot). Two of the three flower types are over 0.100 hexagon units apart (ivory to red = 0.263, red to veined = 0.231). Dyer and Chittka (2004) described a hexagon distance of 0.152 and above as a 'perceptually large colour distance', which bees can learn to discriminate to a high degree of accuracy with absolute conditioning. Smaller colour distances can also be learnt, but by using differential conditioning. Under these conditions, colours as little as 0.045 hexagon units apart can be discriminated. Our data indicate that venation patterns produce a shift in flower colour relative to a red flower that is theoretically visible to the insect eye, but that the difference between veined and ivory flowers is less strong.

The colour shift produced by red veins may be sufficient to explain the prevalence of venation patterns in nature. Alternatively, or in conjunction, there may be close range effects on pollinator landing position and handling of the flower. The molecular mechanism described by Shang *et al.* (2011) for the production of venation patterning involves the combination of a signal from the veins that triggers the expression of *Ve* and epidermal specific expression of *Delila*. Genes encoding bHLH proteins are probably usually expressed in epidermal cells of flowers, since anthocyanins accumulate in this tissue. Consequently the general signal from the veins and the general epidermal expression of genes encoding bHLH proteins regulating anthocyanin biosynthesis might be expected to arise repeatedly in plants with complex genomes containing many duplicated genes encoding transcription factors. If such a pattern enhances

reproductive success by improving flower visibility it is likely to be retained by natural selection in many populations (Shang et al, 2011). Building on these theoretical observations we find that flower-naïve bumblebees can be trained to discriminate between ivory flowers and ivory flowers with red veins, demonstrating that, even if the averaged colours are very similar, the pigmentation pattern is clearly visible to the insect eye. This visibility does not necessarily imply any particular preference for the trait, but indicates that it has the potential to be learned as a cue associated with greater reward or more efficient handling of the flower. Previous studies have shown that increased handling efficiency enhances floral attractiveness to a range of pollinators (Waser and Price, 1985).

Our analysis of innate preferences of flower-naïve bumblebees indicates that both ivory and ivory with red veins are preferable phenotypes in comparison to red flowers. We hypothesise that this result reflects the very dark tone of the red *Antirrhinum majus* line JI522. This inbred laboratory line is much more heavily pigmented than wild varieties of *Antirrhinum majus*, and analysis of its reflectance spectra revealed that the flower reflects very little total light (figure 2a). As well as reduced overall reflection, when plotted into bee visual space (figure 2b), the red flowers are also found to have the least colour contrast with the background. This poor colour contrast can make red flowers harder to find and therefore less preferable (Spathe et al., 2001). Since the red light that the flower is reflecting is only weakly visible to the bee eye, being registered at the tail of the green photoreceptor's response range (Chittka and Raine, 2006), the overall effect will be of a very dark green, hard to distinguish from achromatic soil or green vegetation.

In our array experiment, flower naïve bumblebees demonstrated no preference for veined flowers over ivory flowers. Although the two phenotypes are readily distinguishable under differential conditioning, bees did not innately prefer either line. This result at first seems surprising in light of the relative frequency of veined phenotypes in wild species of *Antirrhinum* (Shang *et al.,* 2011; Wilson and Hudson, 2011). Furthermore, the venation patterns slightly reduce the overall reflectivity of the veined flowers. However, it is the colour contrast of a flower with its background rather than its overall reflectivity that can influence bee preference (Spathe et al., 2001) Given that both ivory and veined flowers plotted in the bee blue-green region of the colour hexagon, and that both reflected a significant amount of light across the range known to excite bee photoreceptors, it is perhaps not so surprising that neither of these relatively similar flowers is immediately preferable to bees. Flower colours that have evolved by natural selection acting through pollinator behaviour (as opposed to those that are the result of genetic drift) might have been selected because they are innately appealing or, alternatively, because they can be learned as useful cues to enhance foraging efficiency. The lack of a significant innate preference for venation in our experiments suggests that the prevalence of this pattern in nature is due to its utility as a guide to the location of the reward, reducing handling time and improving the pollinator's relative energy gained per flower.

The results described here are very different from those reported by Shang *et al.* (2011), using the same veined and red lines of *Antirrhinum majus* and a very similar ivory line. In their study bees preferred red flowers strongly to ivory, and their preference for veined flowers almost matched that of red. However, their experiments were conducted in the field, scoring the choices of experienced wild pollinators. One possible explanation for the differences observed between the studies is that outdoor-grown Antirrhinum flowers contain higher levels of anthocyanins and UV-absorbing flavonols than do the glasshouse-grown plants used here. A further point of difference between the studies is the distance over which bees were foraging, with our experiments analysing short-range choices and those of Shang et al. (2011) comparing long distance foraging decisions. However, that the results of Shang et al. (2011) indicate a significant preference for veined over ivory flowers supports the suggestion that venation is largely a learned morphology, favoured because it directs the pollinator to the nectar reward. This quality would not be immediately apparent to a flower-naïve bee, and so would not be recorded in our experimental design. The preference for red flowers recorded by Shang *et al.* (2011) might plausibly be explained by learning in a similar way. The field in which the experiments described in Shang *et al.* (2011) were carried out

contained many additional plots of red Antirrhinum flowers (Cathie Martin, personal observation). Similarly, wild pollinators in a suburban environment are likely to encounter domestic gardens and many garden varieties are bred for enhanced pigmentation and great depth of colour to the human eye. Accordingly, urban pollinators might become accustomed to dark flowers and learn to associate them positively with nectar and pollen rewards. Alternatively, the preference for red observed in this previous study might also stem from a frequency dependent effect. Bee foraging in a polymorphic population is often observed to be frequency dependent, usually with preference for the more common variety (Eckhart *et al*., 2006). The experimental plots of Shang *et al.* (2011) involved isolated pairwise comparison plots of 64 plants. The plots were separated from the main field of Antirrhinum plants, which were predominantly full red. The proximity (20 metres) of a substantial number of red flowers might have increased foraging from the red flowers in the pairwise plots, or reduced foraging from the other lines. That veined flowers were visited as frequently as red flowers in their red:veined comparison plot argues strongly that the wild bees had learned that venation patterning was a useful foraging guide.

In summary, we conclude that venation patterns of pigmentation are visible to bumblebees and can be learned as a cue to identify rewarding flowers. While previous studies have demonstrated that veined flowers are attractive to experienced foragers, our data demonstrate that flower-naïve bees are not innately attracted to veins. We conclude that venation patterns might be prevalent in nature because they can are learned as useful nectar guides, particularly where they also increase flower visibility.

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Figure Legends

Figure 1. Flowers of the three lines used in this study. Left: red. Centre: ivory. Right: veined.

Figure 2. Optical characterisation of the three flower types. (a) Reflectance spectra of the red (magenta triangles) and ivory (filled grey diamonds) flowers, and of the red part of the veined flowers ("veined red", red circles), the ivory part of the veined flowers ("veined ivory", open diamonds) and of the averaged colour of the veined flowers using a large spot ("veined large spot", pink circles). (b) Positions of the 5 recordings from (a), plus the recording of the brown background, in bee colour space, using the hexagon model of Chittka (1992).

Figure 3. Learning behaviour of bees discriminating ivory from veined flowers, showing the mean percentage of ten visit blocks where bees visited the rewarding flowers. Unfilled bars: ivory flowers rewarding (quinine solution in the veined flowers, 30% sucrose in the ivory flowers); filled bars: veined flowers rewarding (30% sucrose solution in the veined flowers, quinine in the ivory flowers).

Figure 1

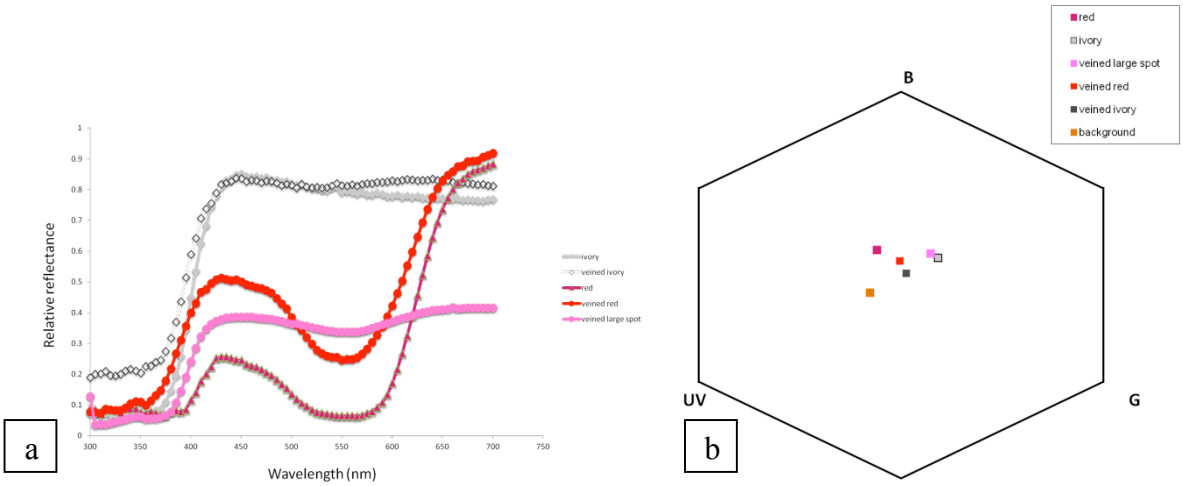
a
Relative reflectance
Wavelength (nm)
ivory
veined ivory
red
veined red
veined large spot
b
B
UV
G
red
ivory
veined large spot
veined red
veined ivory
background

Figure 2

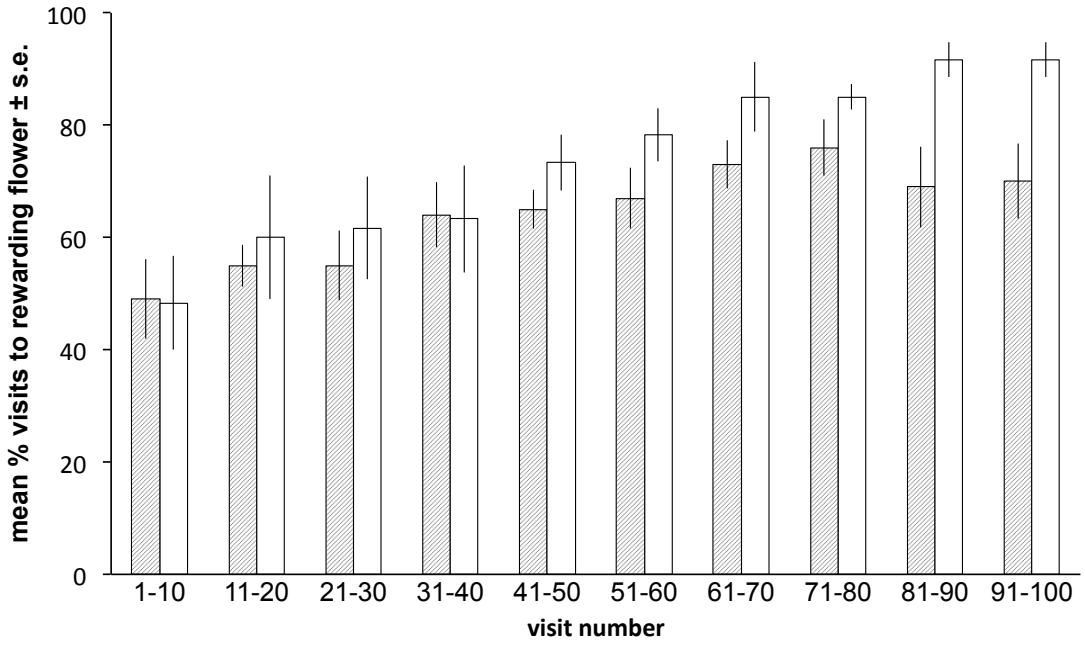
mean % visits to rewarding flower ± s.e.
100
80
60
40
20
0
1-10
11-20
21-30
31-40
41-50
51-60
61-70
71-80
81-90
91-100
visit number

Figure 3